



**Supplementary Fig. 1. Ubiquitination of Ste4 is blocked in a mutant strain that harbors a temperature-sensitive allele of *RSP5* (*rsp5-1*).** Wild type or *rsp5-1* cells were transformed with either empty vector or plasmid expressing His8-tagged ubiquitin (His8-Ubi). Early log phase cells were shifted to restrictive temperature for 4 h (to inactivate Rsp5), treated with 3  $\mu$ M pheromone for 60 minutes. His8-Ubi conjugated proteins were purified from whole cell lysates under denaturing conditions and analyzed by immunoblotting (IB) with anti-Ste4 antibodies to reveal ubiquitinated Ste4. The level of input Ste4 prior to purification as well as the successful purification of ubiquitin conjugates are also shown here.